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Dispatch

Multiciliated cells improve olfaction in aquatic animals

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An elegant new study shows that multiciliated cells in the nose of aquatic vertebrates generate flow fields that help odor detection and processing.

It is intuitively clear to everyone who has ever entered a foul smelling room or conversely sniffed at a rose flower, that the flow of odorants over the olfactory epithelium has a major impact on the sense of smell (olfaction). For land dwelling animals with lungs, breathing provides the regulated flow that suppresses or enhances olfaction.

This option is not available to animals that do not actively breathe. This is true for aquatic animals and in particular for those that dwell in stagnant water.

Evolution has invented a number of ingenious solutions. Many fish display active gill movements flushing their olfactory epithelium, others developed sac-like structures that can actively flush water through the nasal cavity and some even appear to “sniff” by active jaw movements [1,2].

Another mechanism involving non-sensory ciliated cells has been put forward, where motile cilia generate a flow across the olfactory epithelium that is independent from water flow in the environment [3–5].

The elegant study of Reiten et al., in the current issue provides conclusive evidence for such a mechanism, demonstrating that multiciliated cells provide a flow over the olfactory epithelium, directly affecting odor detection and processing [6].

The authors accomplished this by taking full advantage of the optical and genetic accessibility of the zebrafish (*Danio rerio)* larvae. In this commonly used model organism the larval snout contains two nasal pits that are lined with the olfactory epithelium consisting of multiple cell types. The bottom of the pit is lined with olfactory receptor neurons (ORN), each equipped with a few non-motile primary cilia that are decorated with olfactory receptors. The rim is surrounded by a layer of cuboidal multiciliated cells (MCCs) with large bundles of motile secondary cilia protruding. These MCCs are ideally located to generate a flow across the olfactory epithelium.

Since the olfactory pits of larval zebrafish are open to the surface, the beating pattern of the motile cilia can be directly observed in a light microscope. This effort was additionally helped by an enhancer trap line that sparsely labels MCCs in the nose pit with green fluorescent protein. The authors found that cilia of the MCCs generating a flow field around the nose by performing asymmetric, whip like strokes. This complex flow field was beautifully visualised using fluorescent particles and measured by particle image velocimetry. Such measurements established that ciliary beatings generate a stable flow around the snout that draws water medially into the nose pit and ejecting it laterally, leading to a rapid turnover (about twice per second) of fluid filling the nasal pit.

As suggestive as this observation is, the final proof that such fluid field aid in olfaction was still lacking. The authors managed to provide such final prove by unleashing the full power of zebrafish neurogenetics. They used a transgenic line that expresses the calcium indicator dye GCamp6 throughout the entire nervous system, allowing them to optically monitor neuronal activity in the living larvae. By such means they were able to show that odors distanced over 200 afar can be attracted by the flow field and elicit a response in ORNs and the olfactory bulb of the brain. In order to show that this is dependent on ciliary function of MCCs, they made use of the *schmalhans* (*smh*) mutant strain which displays defects in motile cilia due a mutation in the coiled-coil domain containing 103 protein [7]. These mutant larvae cannot generate a flow field and in line with their hypothesis odor molecules are not delivered to the ORNs and consequently no increase in neuronal activity could be measured. Importantly, olfaction in these mutant larvae is unaffected under constant odorant flow that renders the cilia generated flow field unnecessary.

These experiments demonstrate the utility of this cilia generated water flow for stagnant water, but in a naturalistic setting a fish likely encounters dynamic odor plumes, either caused by swimming or water flow. Therefore the authors tested the importance of the cilia generated water flow in the temporal detection of odors by comparing neural responses of wild type and mutant larvae while presenting temporally fluctuating odor plumes. Reassuringly, the response in mutant larvae took longer to elicit the maximum activity at all stages of olfactory procession. Conversely, the dwell time in the nose was longer. In the final experiment the authors systematically varied inter-stimulus intervals between odor pulses and found that sampling was significantly faster and with higher temporal resolution in larvae with functional motile cilia.

Hence the authors closed the circle of their hypothesis by demonstrating that MCCs located around the rim of the nose generate a flow that increases odor detection in stagnant environments and improves the temporal coding of odors in dynamic environments.

A final open question is however how universal this mechanism may be. The author demonstrated similar MCC generated flow fields also in Salmon (*Salmo salar*) larvae. Since the last common ancestor of cyprinids and salmonids lived around 250 million years ago, this result argues that this mechanism evolved a long time ago and is likely widespread among aquatic animals.

But the importance of this MCC generating directed flows over epithelia does not stop here. Recently, MCCs have been identified to play a crucial role in mammals as well [8]. Failure of these cells to generate directed fluid flows across their respective epithelia have been associated with diseases of the respiratory, reproductive system and nervous system [9].

Hence the study of MCCs in the accessible zebrafish may very well not only help us to understand the mechanism of how these fascinating cells generate fluid flow at the cellular and molecular level, but also gain insight into the corresponding human diseases as well.

References

1. Nevitt, G.A. (1991). Do fish sniff? A new mechanism of olfactory sampling in pleuronectid flounders. The Journal of experimental biology *157*, 1–18.

2. Kasumyan, A.O. (2004). The Olfactory System in Fish: Structure, Function, and Role in Behavior. Journal of Ichthyology, S180 - S223.

3. Bashor, D.P., Beuerman, R.W. and Easton, D.M. (1974). Ciliary action and normal movement of odorant wavefronts in garfish nasal capsule of Lepisosteus osseus. Experientia *30*, 777–779.

4. Doving, K.B., Dubois-Dauphin, M., Holley, A., Jourdan, F. (1977). Functional Anatomy of the Olfactory Organ of Fish and the Ciliary Mechanism of Water Transport. Acta zoologica *58*, 245–255.

5. Kux, J., Zeiske, E. and Osawa, Y. (1988). Laser Doppler velocimetry measurements in the model flow of fish olfactory organ. Chemical Senses *13*, 257–265.

6. Reiten, I., Uslu, F.E., Fore, S., Pelgrims, R., Ringers, C., Verdugoa, C.D., Hoffmann, M., Lal, P., Kawakami, K., Pekkan, K., et al. (2017). Motile cilia mediated flow improves sensitivity and temporal resolution of olfactory computations. Current Biology *this issue*.

7. Panizzi, J.R., Becker-Heck, A., Castleman, V.H., Al-Mutairi, D.A., Liu, Y., Loges, N.T., Pathak, N., Austin-Tse, C., Sheridan, E., Schmidts, M., et al. (2012). CCDC103 mutations cause primary ciliary dyskinesia by disrupting assembly of ciliary dynein arms. Nature genetics *44*, 714–719.

8. Brooks, E.R. and Wallingford, J.B. (2014). Multiciliated cells. Current biology : CB *24*, R973-82.

9. Faubel, R., Westendorf, C., Bodenschatz, E. and Eichele, G. (2016). Cilia-based flow network in the brain ventricles. Science (New York, N.Y.) *353*, 176–178.

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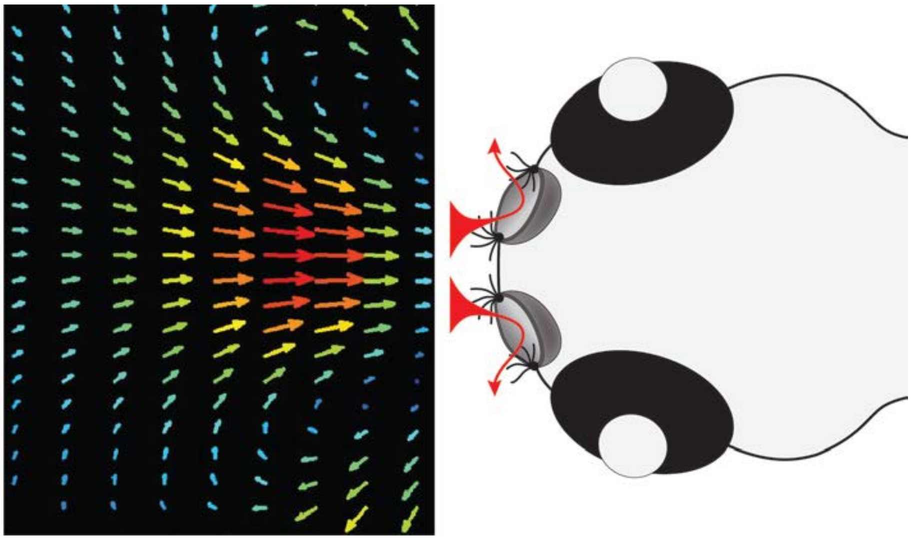

Figure 1. Flow fields generated by multiciliated cells aid olfaction.

Multiciliated cells in the larval zebrafish nose pit beat with asymmetric strokes, generating flow fields around the nose (left panel; warm colors and arrow size indicate flow strength). These flow fields push water mediate into the nasal pit and eject them laterally. This effectively flushes odors over the sensory olfactory epithelium, enhancing odor detection and dynamic odor processing (adapted from Reiten et al., (this issue) with major help from Marion Haug).